

For the 8th International Congress on Molecular Plant-Microbe Interactions;  
July 14-19, 1996; Knoxville, TN:

HARPIN IS NOT NECESSARY FOR THE PATHOGENICITY OF *ERWINIA STEWARTII* ON MAIZE.

Musharaf Ahmad, D. R. Majerczak, and D. L. Coplin\*. Dpt.  
of Plant Pathology, The Ohio State University, Columbus,  
OH 43210-1087, USA.

*Erwinia stewartii* elicits a hypersensitive response (HR) in tobacco if expression of the *hrp*-like *wts* regulon is enhanced. A clone containing *E. amylovora* *hrpNe<sub>s</sub>* was used as a hybridization probe to locate a gene for harpin production, *hrpNe<sub>s</sub>*, within the *wts* gene cluster. Transposon mutagenesis and complementation analysis revealed that *hrpNe<sub>s</sub>* is a monocistronic operon. Sequence analysis indicated that it encodes a 382-amino acid, glycine-rich polypeptide, which lacks cysteine and an N-terminal signal peptide. Harpin<sub>Es</sub> is 58% identical and 78% homologous to harpin<sub>Ea</sub> and 41% identical and 66% homologous to harpin<sub>Ech</sub> from *E. chrysanthemi*. Purified harpin<sub>Es</sub> was protease sensitive and heat-stable, and it elicited a typical HR in tobacco leaves. Antibodies to harpin<sub>Es</sub> cross-reacted with harpin<sub>Ea</sub> and conversely. Harpin<sub>Es</sub> was found in cytoplasmic, membrane, and extracellular fractions. Chromosomal mutations in *hrpNe<sub>s</sub>* were constructed by Tn5 mutagenesis and marker-exchange. The mutants were HR- and did not produce detectable harpin in Western blots. However, they remained fully pathogenic on maize seedlings with respect to symptom severity, ED<sub>50</sub> and response time, and they grew as well as the wild-type strain *in planta*. Likewise, loss of harpin did not affect the ability of a *hrpNe<sub>s</sub>* mutant to grow endophytically in several grasses. *wtsB*, *wtsD*, and *wtsF* mutants accumulated Harpin<sub>Es</sub> intracellularly, indicating that these DNA regions are necessary for harpin secretion.